

Simultaneous Detection of Herpes Simplex Virus 1 and 2 in the Cerebrospinal Fluid of a Patient with Seizures and Encephalitis

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We report a case of a 62-year-old female with seizures and encephalitis. Molecular testing of the patient's cerebrospinal fluid was positive for both herpes simplex virus 1 and 2 (HSV-1 and HSV-2). To our knowledge, this is the first report of simultaneous detection of HSV-1 and HSV-2 in cerebrospinal fluid.

CASE REPORT

A 62-year-old female with a past medical history of breast cancer, rheumatoid arthritis, and atherosclerotic heart disease presented with a rash and vesicular lesions on her shoulder and arm. Due to the distribution of the rash along the 5th cervical (C5) dermatome, she was diagnosed clinically with shingles and treated with oral valacyclovir. After 6 days of treatment, she was admitted to the hospital following a suspected seizure. On physical exam, the previously noted rash was present, though the lesions had crusted over. A neural magnetic resonance image (MRI) performed at this time demonstrated enhancement in the occipital and parietal lobes of the brain. This pattern of neural involvement led to a differential diagnosis of an infectious process, inflammatory process, or posterior reversible encephalopathy syndrome (PRES). Cerebrospinal fluid was obtained, and cell counts demonstrated a lymphocytic pleocytosis (69 white blood cells per μ l, 86% lymphocytes, and 14% monocytes), a glucose level of 70 mg/dl, and a protein level of 60 mg/dl. PCR testing for varicella-zoster virus (VZV) was performed and was negative. Unfortunately, no further testing could be performed due to a limited sample amount. However, the patient dramatically improved while receiving intravenous (i.v.) acyclovir, and she was eventually discharged on oral valacyclovir. Her total treatment course consisted of 9 days of i.v. acyclovir, followed by 10 days of oral Valtrex (1,000 mg, 3 times per day).

Two months later, the patient experienced a second seizure similar to the first. An MRI showed multiple lesions throughout the brain, results worse than the previous findings. Given the widespread involvement and history of breast cancer, there was an initial concern for metastatic disease. In preparation for biopsy, neural imaging was performed 2 days later, only to reveal dramatic interval improvement of the previously seen lesions despite the lack of treatment. Cerebrospinal fluid (CSF) was obtained, demonstrating 2 white blood cells per μ l (87% lymphocytes and 13% monocytes), a glucose level of 61 mg/dl, and protein level of 50 mg/dl. Additionally, a sample of cerebrospinal fluid was sent for real-time PCR testing for herpes simplex virus 1 and 2 (HSV-1 and HSV-2). Real-time PCR was elected for testing rather than viral culture due to its increased sensitivity and more-rapid turnaround time for the diagnosis of central nervous system infection (1, 2). In addition, there was a limited volume of CSF, which precluded the performance of both tests.

The HSV-1/2 real-time PCR was performed using an analyte-

specific reagent (ASR) (Roche Diagnostics, Indianapolis, IN) at Mayo Clinic in Rochester, Minnesota. The HSV-1/2 real-time PCR uses fluorescent resonance energy transfer (FRET) probe technology and is performed on the LightCycler 2.0 (Roche Diagnostics) as previously described (3). It targets the DNA polymerase region of the virus and by using melting curve (melting temperature [T_m]) analysis, the test is able to distinguish between HSV-1 ($T_m = 54^\circ\text{C} \pm 2.5^\circ\text{C}$) and HSV-2 ($T_m = 68^\circ\text{C} \pm 2.5^\circ\text{C}$) infection. A negative extraction control is included with each run to ensure that no DNA contamination occurs during the extraction or amplification phase of testing. The negative extraction control does not contain target nucleic acid but instead contains *Escherichia coli* (ATCC 25922) in stool transport and recovery (STAR) buffer (Roche) at a final concentration of 1×10^2 copies/ml. Testing of the patient's CSF sample was positive with a crossing point (C_p) of 32.69 and two distinct melting curves consistent with the simultaneous detection of both HSV-1 and HSV-2 (Fig. 1). The original CSF sample was tested directly by a second HSV-1/2 real-time PCR (Simplexa HSV-1/2 direct; Focus Diagnostics, Cypress, CA) that is FDA cleared. The Focus direct assay also targets the DNA polymerase region of the virus, though at a different region than that targeted by the Roche ASR, and was positive for both HSV-1 and HSV-2.

The patient was again treated with i.v. acyclovir with eventual transition to oral treatment. Her total course was 10 days i.v. acyclovir, followed by 10 days oral valacyclovir (1,000 mg, 3 times a day) and then suppressive valacyclovir treatment (5 mg per day). Her symptoms improved, and she was ultimately discharged and lost to follow-up. The patient denied any recent or remote history of genital or oral lesions. However, a serum sample was obtained prior to discharge and tested using a HSV type-specific glycopro-

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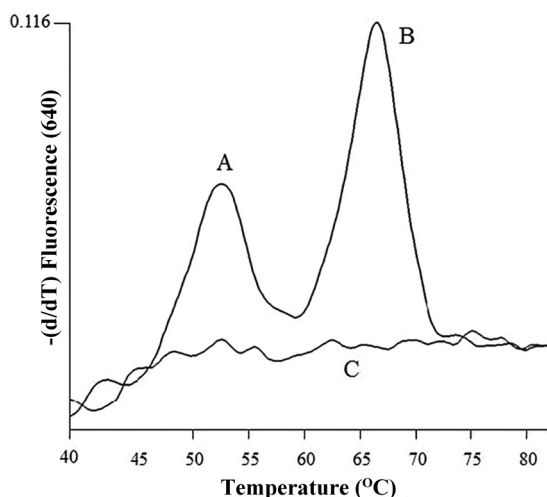


FIG 1 Melting curve analysis of the patient's cerebrospinal fluid specimen showing distinct peaks for HSV-1 (A) and HSV-2 (B). The negative control (C) is also shown.

tein G assay (Bio-Rad BioPlex; Bio-Rad, Santa Venetia, CA). The patient was positive for IgG class antibodies to both HSV-1 and HSV-2, suggestive of past exposure to both viruses.

HSV-1 and HSV-2 are neurotropic, double-stranded DNA viruses. Following infection, these viruses establish latency in sensory neural ganglia and are capable of causing recurrent bouts of disease ranging from cutaneous eruptions to neurologic disease. Central nervous system (CNS) infections secondary to HSV-1 and HSV-2 are well-recognized clinical entities and have been extensively studied. These infections can range in severity from mild and self-limiting to severe and fatal. While there is some overlap in the neurologic manifestations (fever, headache, and confusion) of both infections, significant differences between the two have been described (4–7).

HSV-1 is considered to be the most common cause of sporadic encephalitis, affecting both immunocompetent and immunocompromised patients (7). Since the location of dormancy is usually within the trigeminal ganglia, encephalitis typically arises within the temporal or frontal lobes of the brain first. Brain lesions typically are necrotic and progress rapidly. Symptoms often include altered consciousness, fever, headache, seizure, and personality changes (8). The manifestations of HSV-1 CNS disease can be quite severe with a reported mortality rate as high as 16% and residual neurologic sequelae in up to 62% of patients, despite adequate treatment (5, 6).

In adult populations, HSV-2 usually causes milder disease in the CNS and typically presents as an aseptic meningitis (9). Patients may present with headache, fever, and symptoms of meningeal inflammation (e.g., stiff neck) (5). Cases are usually self-limiting, though treatment with acyclovir is recommended (7). Patients may develop a recurrent lymphocytic meningitis syndrome known as “Mollaret’s meningitis,” characterized by recurrent bouts of meningitis with an increase in atypical lymphocytes within the CSF known as “Mollaret cells.” While encephalitis caused by HSV-2 is rare in the adult population, it is a common cause of neonatal encephalitis. Infection in this population is usu-

ally due to acquisition of the organism through the birth canal of an infected and actively shedding mother. In these patients, the disease progression is rapid and severe, similar to adults with HSV-1 encephalitis.

Since HSV-1 and HSV-2 have different levels of severity in adult populations, several studies have emphasized the need for type-specific testing of CSF samples from symptomatic patients. Several PCR assays that are capable of differentiating between HSV-1 and HSV-2 have been reported (10–12). Subsequent publications have examined the clinical utility of such assays. A 2008 study by Meylan et al. demonstrated significant differences in outcomes between patients that were positive for HSV-1 versus HSV-2 in the CSF (13). A 2003 study by O’Sullivan et al. demonstrated that 89% of patients positive for HSV-1 in CSF had encephalitis, whereas most patients with HSV-2 had meningitis (5).

While many real-time PCR assays have the capability to detect and differentiate HSV-1 and HSV-2, dual infections are rarely described. A 2007 publication by Perkins et al. described a case of genital infection in a pregnant woman caused by both HSV-1 and HSV-2 (14). A large-scale ($n = 8,249$ specimens) retrospective study by Dhiman et al. examined the frequency of dual positivity for varicella-zoster virus and either HSV-1 or HSV-2 (15). They found only a 1.3% dual positivity rate, with dual positive results occurring exclusively for samples from dermal, genital, and oral mucosal surfaces. Pertaining to CNS infections, a study by Ibrahim et al. tested 106 serum samples from patients with encephalitis for a variety of members of the family *Herpesviridae* and found only 2 dual infections (one case of HSV-1 and cytomegalovirus [CMV] coinfection and one case of HSV-1 and human herpesvirus 6 [HHV-6] coinfection) (11). Several prior studies have assessed the prevalence of dual infections in the CSF and did not detect coinfections in these patients (4, 16).

In this report, we describe a patient with seizures and encephalitis who was positive for both HSV-1 and HSV-2 from a single CSF sample. Interestingly, the patient reported no known history of HSV-1 or HSV-2 infection, though studies have demonstrated that up to 82% of patients with HSV-2 CNS infection report no history of genital herpes (5). Due to the rarity of detecting both HSV-1 and HSV-2 in the same sample, we questioned whether amplicon contamination may have caused the dual positivity. However, the CSF sample was tested by two separate molecular methods targeting different regions of the HSV genome, and both assays were positive for HSV-1 and HSV-2. Several publications have described HSV isolates with variant melting curves arising from mutations specific to the probe binding site (3, 17). It is possible that mutations in the probe binding site may yield a melting curve that falls between the expected range for HSV-1 and HSV-2. However, this is rare (<1% prevalence; unpublished data) and does not appear as two unique peaks, as was observed in this case. Further evidence arguing against the possibility that this patient was infected with a single mutant virus is that the specimen was also positive for both HSV-1 and HSV-2 by a second PCR assay, and type-specific IgG serology was performed on a serum sample from this patient and was positive for IgG class antibodies to HSV-1 and HSV-2. Therefore, we feel that this represents the first reported case of dual CNS infection with both HSV-1 and HSV-2.

The exact contribution of each virus to the patient’s clinical status is impossible to determine without more-invasive testing (i.e., brain biopsy). By imaging at her initial presentation, the pa-

tient had MRI findings consistent with encephalitis, which is more commonly caused by HSV-1. However, the distribution of her brain lesions (parietal and occipital lobe involvement rather than temporal lobe involvement) was atypical for HSV encephalitis. Also, at presentation, the patient's cerebrospinal fluid exhibited lymphocytic pleocytosis, more commonly caused by HSV-2. Her primary reason for medical treatment was seizure, a known complication of both viruses. It is also possible that one of the viral subtypes (e.g., HSV-2) was present but not associated with clinical disease. Subclinical reactivation and detection of herpesviruses (e.g., VZV) by real-time PCR has been previously described (18). Regardless, the patient responded to a second course of acyclovir and was ultimately discharged.

This case illustrates that, while rare, dual infections of the CNS with HSV-1 and HSV-2 are possible. Therefore, molecular assays (e.g., real-time PCR) should be capable of detecting both HSV-1 and HSV-2 viruses simultaneously. As multiplex molecular methods become more common in clinical laboratories, it is possible that more coinfections may be recognized, allowing further study of this interesting phenomenon.

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